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Effects of Certain Cadmium Species on Pure and Litter Populations of Microorganisms

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16. ABSTRACT

Cadmium inhibition of mircoorganisms was found to be bacterial and chemical species dependent. E. coli inhibition was a function of the cadmium ion concentration irregardless of the presence of citrate, a chelator for cadmium that it could not metabolize. Whereas with a Pseudomonas sp. able to metabolize citrate, cadmium inhibition was a function of both the cadmium ion and the presence of citrate. With no citrate, inhibition of this organism occurred only at relatively high cadmium ion concentrations (above 10^{-4} M); when citrate was added to the same cadmium-containing growth medium, inhibition was observed at a 1000 times lower cadmium ion concentration, (i.e., 10^{-7} M). This observation is contrary to the classical understanding where a chelate reduces the toxic form of a metal allowing increased growth of the organism. The species of cadmium also differentially inhibited the Douglas fir litter respiration and nitrogen fixing community activities.

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Effects of certain cadmium species on pure and litter populations of microorganisms

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Cadmium inhibition of microorganisms was found to be bacterial and chemical species dependent. *E. coli* inhibition was a function of the cadmium-ion concentration irregardless of the presence of citrate, a chelator for cadmium that it could not metabolize. Whereas with a *Pseudomonas* sp. able to metabolize citrate, cadmium inhibition was a function of both the cadmium ion and the presence of citrate. With no citrate, inhibition of this organism occurred only at relatively high cadmium-ion concentrations (above 10^{-4} M); when citrate was added to the same cadmium-containing growth medium, inhibition was observed at a 1000 times lower cadmium-ion concentration (i.e., 10^{-7} M). This observation is contrary to the classical understanding where a chelate reduces the toxic form of a metal allowing increased growth of the organism.

The species of cadmium also differentially inhibited the Douglas fir litter respiration and nitrogen-fixing community activities.

INTRODUCTION

Deleterious biological effects in soils resulting from the application of heavy metal containing materials such as sludges (CAST, 1976; Sommers, 1977), fertilizers (Williams and David, 1973; 1975), rubbish (Rånby, Albertson and Jansson, 1978) and fallout from flyash (Ratsch, 1974; Natusch, 1978) have been observed both in the laboratory (e.g., Bond et al., 1976; Lighthart and Bond, 1976; Lighthart et al., 1977*a*,*b*) and nature (e.g., Rühling and Tyler, 1974; Watson et al., 1976). Although the mechanism(s) of these effects is (are) poorly understood, it is thought that metals form an array of ionic and complex chemical species (e.g., Volk and Lighthar, 1978) with the soil components, any of which may be a biological toxic agent (e.g., Sarsfield and Mancy, 1975; Sunda and Guillard, 1976; Sunda, Engel and Thuotte, 1978; Sunda and Lewis, 1978).

Reviews of heavy-metal effects on microorganisms are given in Gadd and Griffiths (1978) and Babish and Stotzky (1978).

This discussion presents results of research that was designed to observe the effects on growth of cadmium, both ionic and complexed with citrate, using pure cultures of *Escherichia coli*, a relatively cadmium-sensitive citrate non-utilizing organism, and a *Pseudomonas* sp., a relatively cadmium-resistant citrate-utilizing isolate; and microbial populations in Douglas fir litter where effects were measured as changes in community respiration and nitrogen fixation.

Methods

Organisms

The *E. coli* (ATCC 1795) used was comparatively cadmium sensitive and unable to metabolize citrate as a sole carbon source. The *Pseudomonas* sp. was enriched from sewage sludge and soil inoculated into a cadmium-containing nutrient broth (DIFCO) solution. The *Pseudomonas* sp. was comparatively cadmium resistant and able to utilize citrate as a sole carbon source.

Test growth medium

Both organisms were grown in a high phosphate concentration synthetic medium consisting of glucose (1.76×10^{-2} M), NH_4Cl (9.91×10^{-3} M), NaCl (8.56×10^{-3} M), $MgSO_4$ (2.03×10^{-4} M), $FeCl_2$ (6.16×10^{-5} M), KH_2PO_4 (4.13×10^{-3} M), pH 7.2 HEPES buffer (acid form; 4.49×10^{-2} M, basic form; 1.51×10^{-2} M; Cal. Biochem., San Diego, CA) for several generations prior to inoculation into the test medium which consisted of the high phosphate concentration medium, with only 4.13×10^{-6} M KH_2PO_4 and either background, 10^{-5}, 10^{-4}, 10^{-3}, or 10^{-2} M $CdSO_4$ with or without 10^{-4} M sodium citrate. The cell suspension of a twice saline (0.85% NaCl) washed and suspended inoculum was introduced into 15 ml quantities of medium in duplicate 18 × 120 mm screw-cap test tubes to an opacity of 0.2 as measured on a Spectronic-20 spectrophotometer set at 660 nm. (Growth was measured as optical density minus initial optical density.) Incubation was at 25 °C in a to-and-fro 45° angle shaker running at 75 oscillations per minute.

Microcosms

Two sets of 8 microcosms containing Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) needle litter (23.4 g dry wt) were prepared similarly to Lighthart et al. (1977*b*) and Lighthart and Volk (unpublished results). Into 9-day-old pairs of microcosms, 2 ml of aqueous 5 mM $CdSO_4$/kg of litter, or 2 ml of 10 mM aqueous sodium citrate/kg of litter, a mixture of both or neither was injected into the litter at 48 locations. Respiratory carbon dioxide evolved from the microcosms was measured by the classical alkali-trap-acid titration method (Colé-

man, 1973) in the first set of microcosms. In the second set, nitrogen-fixation (as acetylene reduction) was measured similarly to Fishbeck, Evans and Boersma (1973). Controls for background ethylene production in the microcosms and ethylene-contaminated acetylene gas were prepared.

Cadmium-ion measurement

The cadmium-ion content of the culture solutions was measured using an Orion Research model 910 specific-ion meter with an Orion cadmium (94–48A) – calomel electrode pair.

Results and Discussion

Results of this study will be discussed as two topics. The first topic concerns the effects of cadmium speciation on pure cultures of *Pseudomonas* sp. and *E. coli*.

The growth of *Pseudomonas* sp. on the minimal phosphate test medium was much greater than growth of *E. coli* (Fig. 1, compare *E. coli* and *Pseudomonas* sp.

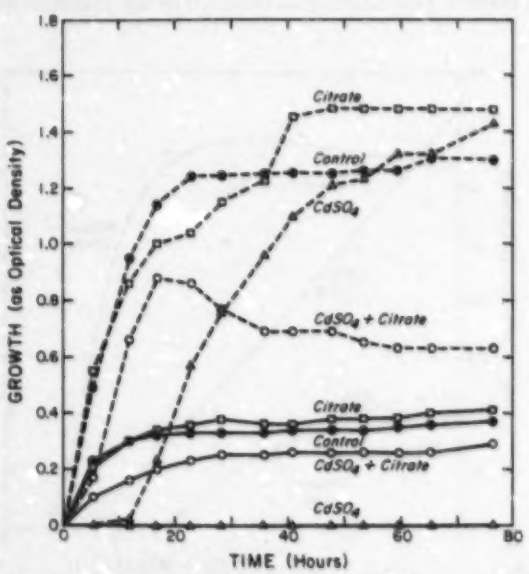

Fig. 1. Growth of *Escherichia coli* ATCC 1795 (——) and *Pseudomonas* sp. (- - -) in a defined medium amended with either 10^{-4} M cadmium sulfate (△), or 10^{-3} M sodium citrate (□), or both (○), or neither (●).

control curves). After adding citrate to the minimal medium, growth was as expected, i.e., *E. coli* was unaffected (Fig. 1, compare *E. coli* curves for citrate and control) while the *Pseudomonas* sp. showed growth in a diauxie-like pattern (Fig. 1, compare *Pseudomonas* sp. citrate and control curves before and after 18 h) indicating initial glucose and subsequent citrate utilization. Addition of cadmium alone (as $CdSO_4$) did not inhibit the *Pseudomonas* sp. until approximately 10^{-4} M cadmium ion was measured in the culture solution (Fig. 2). *E. coli* inhibition occurred in 1/10th to 1/100th as much ionic cadmium (Fig. 1, compare $CdSO_4$ curves for both organisms, and Fig. 2, compare *Pseudomonas* sp. and *E. coli* curves without citrate).

Addition of sodium citrate to the minimal medium affected the growth of the organisms in two ways: 1) It reduced the free cadmium ion (see Sillen and Martell, 1971, for the stability properties of this complex) by complexation with the citrate moiety thus effectively reducing the toxic ionic forms of the medium for the *E. coli* (Fig. 2, compare *E. coli* points with and without citrate); and 2) although the free cadmium ion was reduced by the citrate, there was a marked increase in the sensitivity of the *Pseudomonas* sp. (Fig. 2, compare *Pseudomonas* sp. cadmium curves with and without citrate). That this sensitivity is associated with the citrate moiety is suggested by the fact that when citrate began to be metabolized, marked growth changes occurred in the *Pseudomonas* sp. culture

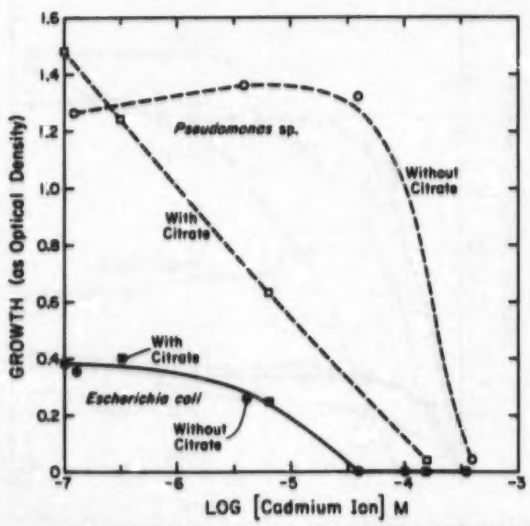

Fig. 2. Sixty-hour growth (as optical density) of *Escherichia coli* ATCC 1795 and *Pseudomonas* sp. in a defined medium, with and without 10^{-3} M sodium citrate, and the indicated measured initial cadmium-ion concentration.

(Fig. 1, compare *Pseudomonas* sp. citrate with the control curves for presumed citrate utilization, and citrate with citrate + $CdSO_4$ for time of inhibition of growth). It is emphasized that, contrary to the classic circumstance where addition of a metal chelator results in decreased inhibition presumably due to the metal forming a non-toxic complex with the chelate (e.g., see Gadd and Griffiths, 1978, p. 305), in this instance addition of a metal chelator markedly and unexpectedly increased metal inhibition.

Inhibition by a metal chelate that is also a substrate is herein defined as 'chemically complexed metal – substrate inhibition'. The mechanism for metal – substrate inhibition may lie in the ability of the cell's membrane and wall to adsorb and thus exclude ionic-metal species such as found by Tornabene and Edwards (1972) for lead bromide in *Micrococcus luteus* and *Azotobacter* sp., or, in the ability of the more liposoluble metal-chelate species to penetrate the cell membrane. After penetration, the substrate portion of the complex may or may not be metabolized, allowing cadmium to react with and thus disrupt the cellular machinery, for example, by reacting with sulfhydryl groups of proteins, etc.

The implications of this phenomenon suggest that those organisms exposed to a toxicant such as cadmium that are unable to metabolize any of the complexed species may only be inhibited by the ionic form whereas those organisms able to metabolize the complexing moiety as a substrate may potentially be inhibited to a greater extent by the complexed species. For example, if a soil has a low organic content one might expect relatively few nutrient metal chelates and relatively high ionic-metal species levels. Therefore, those organisms with ionic-metal sensitivity will be inhibited whereas in rich organic soil, substrate metal chelates might be expected in high concentration and one might anticipate greater inhibition of those organisms able to metabolize the substrate. Consequences of the inhibition of soil microorganisms might be to reduce the rates of organic solubilization and mineralization, i.e., decomposition. Problems arising in soils treated with heavy-metal sludges and then subjected to acid precipitation may be particularly troublesome.

The second aspect of this discussion relates to the laboratory microcosms with Douglas fir needle litter. In these microcosms, nitrogen fixation and respiration averaged 2.13 mg CO_2/g dry wt and per day and 3.67×10^{-2} mg C_2H_4/g dry wt and per day*, respectively, with a higher rate during the initial 14-day flush (see Table 1). Except for a 6% increase in respiratory activity when citrate was added to the litter, all treatments reduced respiration and, particularly nitrogen-fixation activity in the microcosms. Community nitrogen fixation was inhibited more by the ionic-cadmium treatment than by the complex species, i.e., 36 and 19%, respectively, compared to the control. Conversely, inhibition of community respiration was greater with the complexed species than by ionic cadmium, i.e., 7 and 4% compared to respiration of the control microcosms.

*The coefficient of variation for this measurement has been estimated at 1.6%.

Table 1. Comparison of the mean total nitrogen fixed, and the carbon dioxide respired in pairs of 'identically' prepared and incubated Douglas fir (*Pseudotsuga menziesii*) needle litter microcosms amended with 2 ml of 5 mM cadmium sulfate per kg dry wt soil, or 2 ml of 10 mM sodium citrate per kg dry wt soil, both, or neither.

Treatment		Nitrogen fixed		Carbon dioxide respired	
Cadmium	Citrate	Total (10^{-3} mg C_2H_4/mg dry wt·d)	% of control	Total (mg CO_2 /g dry wt·d)	% of control
–	–	3.67	100	2.13	100
–	+	3.48	95	2.26	106
+	–	2.36	64	2.04	96
+	+	2.99	81	1.98	93

These experiments indicate that community activities, e.g., respiration and nitrogen fixation, are not only sensitive to cadmium but also react differently to the various chemical species of cadmium. The same might be true for other metals.

To predict the impact of land application of toxic metal pollutants on life forms such as soil bacteria, it is apparent that managers must understand the species chemistry of the system involved.

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